EFFECT OF DEXTRAN SULFATE ON FIBRONECTIN–COLLAGEN INTERACTION

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1. Introduction

Fibronectin, a high molecular weight glycoprotein present in the blood and extracellular matrix interacts with other components of the extracellular matrix. It binds to collagen [1,2] and interacts with cell surfaces through another, independent binding site [3] mediating attachment of cells to collagen in vitro [4,5]. Fibronectin has also been found to interact with heparin [6]. The binding of fibronectin to collagen allows its isolation by affinity chromatography on columns of gelatin–Sepharose [1]. The fibronectin–gelatin complexes in such columns interact with glycosaminoglycans such as heparin, and, less strongly, with heparan sulfate and hyaluronic acid, but not with chondroitin sulfates. The interaction of glycosaminoglycans with fibronectin bound to gelatin–Sepharose stabilizes the complexes as evidenced by the fact that higher concentrations of urea are required to dissociate complexes treated with glycosaminoglycans than is the case without such treatment [7,8]. Since these interactions are potentially important in the understanding of the formation of extracellular matrix, we have carried out further studies on the specificity requirements involved.

We report here that dextran sulfate shows a strong interaction with fibronectin bound to gelatin–Sepharose particles and competes with heparin for binding to such particles.

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2. Material and methods

2.1. *Polysaccharides*

Dextran sulfate (mol. wt 500 000 and 40 000, sulfate content ~50%), heparin (porcine intestinal mucosa, grade I), hyaluronic acid (human umbilical cord, grade I), chondroitin sulfates (whale cartilage, type A; pig skin, type B (dermatan sulfate); shark cartilage, type C; whale cartilage, mixed isomers, grade III), and calf thymus DNA (type I) came from Sigma (St Louis, MO). ^{35}S-labeled heparin was from Amersham (Arlington Heights, IL).

2.2. *Assays*

Gelatin–Sepharose columns [1] were used to isolate fibronectin and to study the effect of polysaccharides on the binding of fibronectin to such columns. Columns (2 ml) of gelatin–Sepharose were saturated with fibronectin by equilibriating the columns with 15 ml human plasma. After washing with phosphate-buffered saline (PBS), one column volume of solution of polysaccharides in PBS or PBS alone, were passed through the column. After the polysaccharide solution had entered the column, the flow was stopped for 5 min. The flow was then resumed and the column washed and eluted by applying urea or salt in progressively higher concentrations using two column volumes of each solution. Elution of fibronectin was monitored by measuring A_{280}.

Binding and inhibition of binding of ^{35}S-labeled heparin to fibronectin was studied in a tube assay in which ^{35}S-labeled heparin (4.5×10^4 cpm, 0.25 μg, in 1 ml PBS) with or without unlabeled polysaccharide

was incubated with fibronectin-loaded gelatin–Sepharose particles prepared as above for columns of gelatin–Sepharose. After incubation at room temperature for 16 h with continuous mixing, the particles were washed 3 times with PBS. The bound radioactivity was eluted with 2 ml of a solution of 8 M urea in 2 M sodium chloride and an aliquot was counted in a scintillation counter. In some assays, fibronectin coupled directly to Sepharose was used instead of fibronectin bound to gelatin–Sepharose. Fibronectin–Sepharose was prepared by coupling 10 mg human plasma fibronectin [1] to 1 g cyanogen bromide-activated Sepharose (Sigma, St Louis, MO) according to the manufacturer's directions. Over 90% of the added fibronectin became bound under these conditions.

3. Results

3.1. *Effect of dextran sulfate on the elution of fibronectin from gelatin–Sepharose*

We have shown recently that heparin stabilizes complexes formed between fibronectin and gelatin in gelatin–Sepharose columns against dissociation with urea [7,8]. This stabilizing effect was even stronger for dextran sulfate of mol. wt 500 000 (fig.1), while the 40 000 mol. wt dextran sulfate had an effect equal to that of heparin. In the presence of 50 mM Tris–HCl, 2–3 M urea caused elution of fibronectin from columns not treated with dextran sulfate, while not even 8 M urea was sufficient to cause elution of fibronectin from columns treated with the 500 000 mol. wt dextran sulfate. A combination of high concentrations of urea and salt brought about a complete elution of fibronectin. High concentrations of sodium chloride without urea did not cause any elution of fibronectin irrespective of whether or not the column had been treated with dextran sulfate (not shown). The effect of ionic strength on the elution of fibronectin from columns treated with dextran sulfate by urea was studied more closely. While 3 M urea eluted ~70% of fibronectin from untreated gelatin columns at 50 mM salt, 150 mM salt was required for 3 M urea to elute any fibronectin from columns treated with dextran sulfate (fig.2). Higher concentrations of urea partly compensated for the requirement of salt, but the dextran sulfate-treated columns always required a higher concentration of salt for a given concentration of urea and a higher concentration of urea for a given concentration of salt.

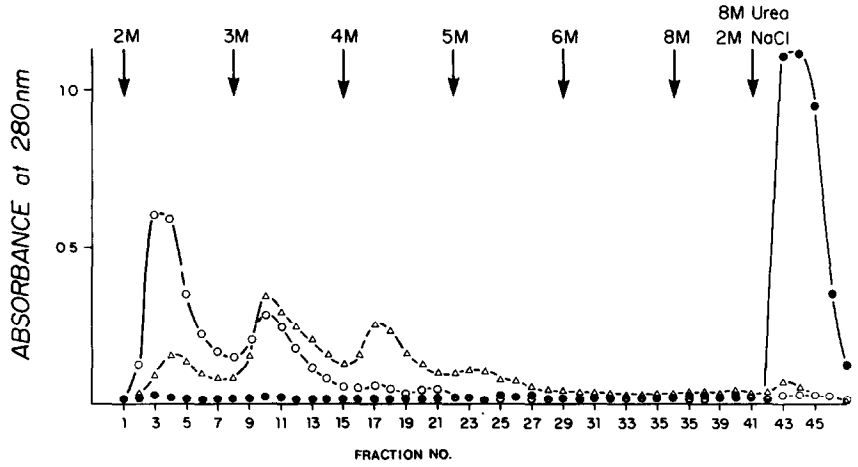

Fig.1. Effect of treatment with heparin and dextran sulfate on the elution of fibronectin bound to gelatin–Sepharose. Fibronectin from 15 ml human plasma was bound to 3 identical columns (2 ml) gelatin–Sepharose. After washing, 2 ml of a solution of polysaccharide (2.5 mg/ml) or PBS was passed through the columns. After further washing with 50 mM Tris–HCl buffer (pH 7.0) the columns were eluted by applying increasing concentrations of urea in the Tris–HCl buffer, and 1 ml fractions were collected. The amount of fibronectin eluted was monitored by absorbance. (○) PBS-treated control column; (△) column treated with heparin; (●) column treated with mol. wt 500 000 dextran sulfate.

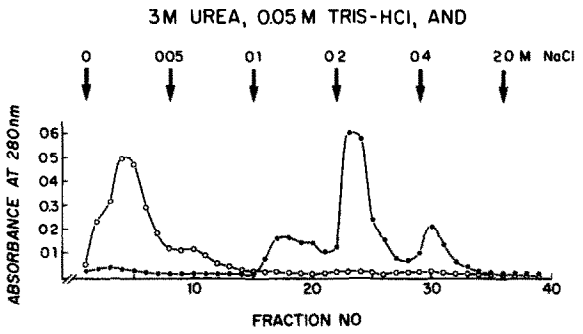

Fig.2. Effect of treatment with dextran sulfate on the elution of fibronectin bound to gelatin–Sepharose. Columns similar to the ones described in the legend to fig.1 were eluted with 3 M urea in 50 mM Tris–HCl buffer (pH 7.5) at increasing concentrations of NaCl as indicated. (○) PBS-treated control column; (●) column treated with mol. wt 500 000 dextran sulfate.

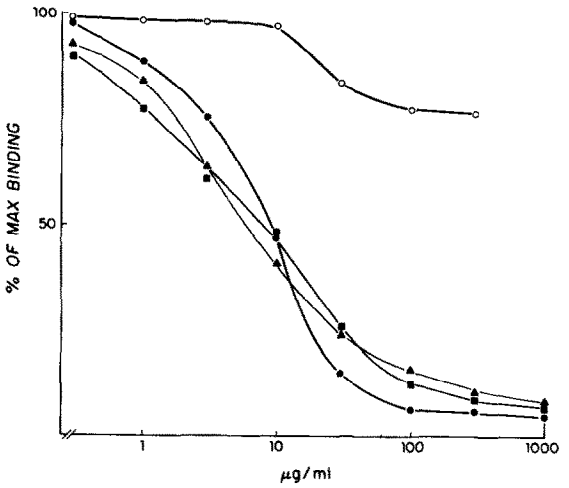

Fig.3. Inhibition of binding of ^{35}S-labeled heparin to gelatin–Sepharose (0.3 ml) loaded with fibronectin by: (▲) heparin; (●) mol. wt 500 000 dextran sulfate; (■) mol. wt 40 000 dextran sulfate; (○) heat denatured DNA (100°C, 15 min). Ordinate: Percent of maximal binding of ^{35}S-labeled heparin. Abscissa: Concentration of inhibitor tested.

3.2. *Binding of ^{35}S-labeled heparin to fibronectin and gelatin–Sepharose and its inhibition by polysaccharides*

Up to 40% of the radioactivity in commercial ^{35}S-labeled heparin can be bound to gelatin–Sepharose loaded with fibronectin [8]. To study the specificity of the interaction of dextran sulfate with fibronectin, an inhibition assay based on this binding was used.

The binding of ^{35}S-labeled heparin to gelatin–Sepharose loaded with fibronectin was inhibited by unlabeled heparin and could also be inhibited by the dextran sulfates (fig.3). The latter were approximately as active as heparin in this assay. DNA, another polyanion, showed weak inhibitory capacity. Chondroitin sulfates A and C, dermatan sulfate, and hyaluronic acid were not significantly inhibitory (not shown). Similar results were obtained when particles where fibronectin was coupled directly to Sepharose were used.

4. Discussion

We found dextran sulfate to share with heparin the property of interacting with fibronectin and fibronectin–gelatin complexes. Our results allow some conclusions with regard to the specificity and structural requirements of the interaction of glycosaminoglycans and other polysaccharides with fibronectin and fibronectin–collagen complexes. The dextran sulfate preparation with mol. wt 500 000 was more active than either the 40 000 mol. wt preparation or heparin in strengthening the binding of fibronectin to gelatin–Sepharose columns. This suggests that the stability of the complexes formed may increase with the increasing molecular weight of the polysaccharide.

The binding of glycosaminoglycans to fibronectin apparently is dependent on ionic interactions, but also seems to involve structural specificity in the polysaccharide beyond polyanionic properties. In spite of a sulfate content of ~50%, the dextran sulfates were not more active than heparin (28% sulfate) in competing with ^{35}S-labeled heparin for the binding to fibronectin. However, it seems that the activity may at least partly depend on the degree of sulfation, because we have shown that a heparan sulfate subfraction with only 9% sulfate does not detectably interact with fibronectin, and another subfraction with 17% sulfate is active, although less so than heparin [8].

Both heparin [9] and dextran sulfate [10–12] have been reported to inhibit the growth of transformed cells, and in the case of dextran sulfate, to alter their morphology toward normal [13]. Trans-

formed cells, while they do produce fibronectin, generally lack the cell surface-associated fibronectin matrix that normal cells possess (see [14]). It will be interesting to see whether the effects of dextran sulfate on transformed cells are mediated through changes in the formation of the fibronectin matrix.

The interaction between glycosaminoglycans, fibronectin, and collagen may play an important part in the assembly of extracellular matrices. The significance of these interactions to the normal formation of extracellular matrix and disturbances of this process remains to be studied, but the recent results in [15] suggest that fibronectin is closely associated with proteoglycans at the surface of cultured cells.

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